THE ORIGIN AND NATURE OF THE MUCILAGE IN THE CACTI AND IN CERTAIN OTHER PLANTS*

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The increasing interest in the rôle of emulsoids in the economy of the plant, especially in that of the growth processes, has indicated the necessity of re-examining them from the more recently taken point of view exemplified in the work of Borowikoff, Long, MacDougal, Spoehr, and others.[1] More specifically regarded, the question of the relation of alterations in the volumes of emulsoids due to changes in the acid and salt content of the tissues, and the effect of these alterations on the volume of the plant body is one of paramount importance, and is particularly to the fore at the moment.[2]

In a good many plants the presence of very considerable quantities of mucilages and "gums" has of course long been a matter of common knowledge and has been much investigated, chiefly from the point of view of the histologist and pharmacologist[3] with only side glances at their physiological contacts, leaving much to be said from the present point of view. The following account is confined principally to the mucilage of Opuntia *in situ*, having regard to its origin and distribution, coupling therewith some notes on the comparable conditions found in Tilia, Malva, and *Astragalus gummifer* (tragacanth).

DISTRIBUTION OF MUCILAGE CELLS

The mucilage in Opuntia originates within, and is normally confined to, definite large cells (mucilage idioplasts) scattered throughout the parenchyma, both medullary and cortical. Their absolute number is correlated with the species. Their distribution in the cortex and medulla differs also in different species. Thus, in *Opuntia* sp. (a species obtained from Dr. D. Griffiths and now in the garden at the Coastal Laboratory of the Carnegie Institution of Washington) none are found within 2.5 mm. of the epidermis of the joints, though they occur more closely thereto in the leaves. In *Opuntia Blakeana*, however, they lie very near the epidermis. For this reason it is practically possible, in the former species, to cut sections of the chlorenchyma parallel to the epidermis which when allowed to lie in water give off no mucilage. There is observable no mucilage identical with that of the mucilage idioplasts in any other cells, though it is not at the moment denied that there may be a very small amount of hydrophile colloid in the vacuoles of the ordinary parenchyma cells.

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[1] For citations see MacDougal and Spoehr, 1917.

[2] MacDougal and Spoehr (*l. c.*); Lloyd, 1917, 1918*a*.

[3] See Tschirch, 1884.

The number of mucilage cells in the cortex may be smaller per unit volume of tissue than in the medulla, but on the other hand there is frequently realized a condition in which both cortex and medulla are crowded with them. In a joint of *Opuntia susquehannensis* about 3.5 cm. long, and which, though small, was shrunken as if it had been long deprived of water, the mucilage cells were so large and numerous as to occupy much more than half the total volume of the whole. They were moreover crowded upon each other to such an extent as to approximate a lacunar condition such as Walliczek (1893) described for Tilia. Trécul (1875) believed this to be true also for cacti, etc., but in view of the peculiar difficulties of observation a reasonable question of fact may be permitted.

Place and Time of Origin

Though occurring within both medulla and cortex, the mucilage cells arise first in the medulla and later in the cortex. The earliest may be found in the medulla directly beneath the growing point, while in the cortex the youngest readily recognizable as passing into the more obvious condition of a definitive mucilage cell could be found only as far as 4 mm. from the apex, in the Opuntia above mentioned. Relatively few, however, originate within the actively expanding region at the apex of a young joint. They arise rather during the whole period of growth in all regions of the enlarging joint, young ones being found even toward the base of a joint several centimeters long. They are therefore secondary in their origin, and before they assume their special character are indistinguishable from the surrounding cells, whether of cortex or medulla. It is a legitimate speculation that the numbers of mucilage cells may be modifiable under various environmental conditions.[4] In *Carnegiea gigantea* the mucilage cells are not to be found in the palisade tissues and are considerably fewer in number than in Opuntia. They are absent from the younger tissues, none being found by me in a small individual 45 cm. tall, except below the level of *ca.* 30 cm. from the apex.

Mode of Origin, Chemical Structure and Inclusions

The mucilage cells as such are at first recognizable only by their size. When once differentiable, one notes that the nucleus and nucleolus enlarge to an enormous size. At first parietal in position, the nucleus usually becomes central. The protoplasm also increases in amount both absolutely and relatively, and the nucleus becomes suspended in many thick strands. Chloroplasts and starch grains are usually present, and a large stellate cluster of calcium oxalate crystals is frequently, though not invariably, to be seen.

The wall is at first indistinguishable from the walls of surrounding cells, but when considerable size has been gained it becomes somewhat thickened.

[4] According to Tschirch (*fide* Walliczek, 1893, p. 274), the mucilage content of the marshmallow is greater when the plant is grown in dry soil.

So long as the cell has not passed beyond the condition thus far described, the addition of water to fresh or alcohol-fixed material does not affect its internal topography, whereas when the secretion of mucilage has been begun this is not the case. Assuming however the contrary, that mucilage has already appeared, and providing that the cell is still immature, the imbibition of water from the surrounding *milieu* by the mucilage causes a displacement of the protoplast from the cell wall more or less complete.[5] This is explained by the circumstance that material capable of a high degree of hydration now occupies the inner surface of the cell wall. It can now be shown that an inner zone of this wall, approximately half as thick as the whole, is in a more hydrated condition than the remaining cell-walls because it *gives a deeper blue coloration with iodine.* The inner face of this zone is not optically definable but fades into a colorless substance, the mucilage. The amount of this present is indicated by the amount of displacement of the protoplast from the wall. Usually the protoplasm will hold to the wall at several points, especially where pits occur, and when the mucilage becomes more abundant its swelling results in the somewhat bizarre appearance of an entire protoplast compressed at the middle of the cell and connected by strands of protoplasm with the wall at several or many points. The larger conspicuous strands have their distal place of attachment to the cell wall at or very near the middle points of the areas of contact of the contingent parenchyma cells. This appears clearly to indicate that the reason for adherence is the presence of the intercellular connections at these points, which are marked by wide, shallow pits.

When the amount of mucilage arrives at or near to the maximum, the imbibition of water permits its hydration to such an extent that the protoplast becomes crowded into an irregular echinate mass, the radiating protoplasmic processes being either detached from the wall or variously torn asunder. The mucilage itself is now seen, but with some difficulty, to be laminated, the zonation being parallel to the cell wall but with curvatures toward the pits. This zonation, seen in *Opuntia elatior* by Cramer (*fide* Wigand, 1863, p. 149), is due to varying degrees of hydration (Walliczek, 1893) as appears from the fact that, when dehydrated with alcohol, internal syneresis occurs much more extensively in the more hydrated zones, which are then discoverable to the eye as zones of small spherical cavities of various sizes, but all minute. Such syneretic cavities may, however, be quite large, depending, in part at least, on the rate of dehydration, and probably also on the degree of hydration of the mucilage as a whole. The lamination is generally observable before or during the course of swelling, and is much more evident in *Carnegiea gigantea.* In this form the mucilage swells more slowly, and the loss of the marked lamination during increasing hydration

[5] In order to form a critical judgment of the condition of the mucilage cells, fresh sections must be examined without the addition of water, as should also fresh and alcohol-dehydrated material with added water.

may be readily followed on the addition of water. The lamination in tragacanth, seen by Kützing (*fide* Wigand, 1863), is still more evident, and is lost only very slowly at ordinary temperatures, while that of Sterculia appears to be still more resistant (Maiden, *fide* Tschirch, Lehrbuch, p. 403, vol. 2, pt. 1).

If mucilage cells in this condition are cut open in the making of a section, the mucilage swells enormously on the addition of water, oozes out from the cell cavity and, carrying the protoplasm and its inclusions with it, forms a rope. If pieces of tissue are placed in water, they gradually become translucent. This is due to the expulsion of air from the intercullular spaces, resulting partly at any rate from the bursting *in situ* of the mucilage cells. This fact may be demonstrated by examining sections of a piece of tissue which have lain in water, the sections being dehydrated and examined in alcohol. In the case of the medulla of a frond several centimeters thick, in which the elongation of the cells had taken place in a direction normal to the surface of the frond, it was found that the bursting of the mucilage cells had taken place in this direction. This, it was evident, was due to the mechanical conditions offered by the web of cell walls and the mutual pressures of the cells. Whether such bursting occurs within the plant in consequence of local disturbances, resulting in the extrusion of mucilage into the intercellular spaces, and possibly filling lacunae (schizogenous or lysigenous, or due merely to tearing) within the tissues, is not proved, though it is rather to be expected, especially when the mucilage cells are large, numerous, and mutually contingent. It may be noted in passing that in some species of Opuntia and in other genera (*e. g.*, Ariocarpus) there are lysigenous canals or lacunae filled with a gummy secretion, but of a different nature from that being here considered.

Effect of Anaesthetics

Dr. H. A. Spoehr pointed out to me that an abundant oozing of mucilage takes place on treatment of tissue with choloroform, ether, etc. I offered the explanation that the immediate effect of the reagent was to asphyxiate the parenchyma cells by which the mucilage cells are surrounded, upon which they give up their water into the intercellular spaces, making it possible to hydrate the mucilage cells. This was verified as follows.

A section was placed without the addition of any medium on a cover glass and inverted over vapor of ether in a small glass cell. In the course of a minute, the air in the intercellular spaces began to be expelled by water escaping from the parenchyma cells. It could then be clearly seen that the mucilage cells became more hydrated, as was proved to the eye by the further displacement of the protoplast. Radial strands reaching to the cell wall could be observed in the breaking, and the whole mass of protoplasm to be further crowded toward the middle of the cell. In some instances the cell walls were broken, and the mucilage could then be seen oozing out therefrom

through circular perforations, recalling the similar behavior in the tannin idioplasts of the persimmon (Lloyd, 1911). In the course of time the parenchyma cells completely collapsed, and the mucilage had then reached its maximum hydration permissible under the circumstances. The preparation was now stained in alcoholic safranin and the few mucilage cells remaining unbroken were stained. On being placed in water nearly all of these subsequently burst under microscopic observation.

Inclusions in Mucilage Cells

Starch is generally found within the protoplast *sensu stricto*, in amounts usually correlated with the amount found in the neighboring cells, but sometimes in less quantity.[6] It is perhaps unexpected to find that in a much shrunken frond of *Opuntia susquehannensis*, already referred to, the old and fully hydrated mucilage cells contained a very large amount of starch, as did the remaining parenchyma. No evidence of an inverse quantitative relation between the amount of starch present and the extent of mucilage secretion could be observed. This starch, it would seem wholly probable, was laid down after the mucilage had been secreted. This view suggests the question of the physiological condition of the mucilage cells after the amount of mucilage is sufficient to cause displacement of the protoplast from its usual and conceptually normal position, namely, against the cellulose wall. Specifically, does the protoplast become moribund and eventually die when compressed within the swollen mucilaginous mass? Without attempting at the moment to answer this question, it may be pointed out as bearing on it, that the size of the mucilage cell does not appear to remain fixed after the amount of mucilage has become sufficient to press the protoplast into a relatively small compass within its interior. It is certain, at any rate, that there is no disappearance of mucilage cells during the developmental phase of the frond, and it is similarly certain that the size of these cells in the mature tissue is much greater than in young, *quasi* embryonic material. Thus, in a frond some centimeters long, the mature mucilage cells near the growing tip measured 0.15 mm. in diameter. Toward the middle of the frond they measured fully 0.5 mm. in diameter, a gradual increase in size being observable as the eye receded from the growing apex. In view of the possible secretion of starch above mentioned, it seems possible that in spite of the crowding of the protoplast by the hydrated mucilage, it remains alive, and that the cell grows. In this event, the pressure on the cell wall which causes stretching is dominantly the imbibition pressure of the mucilage.

General Discussion

A review of the literature pertaining to the matter under present treatment shows clearly that the essential features of the topography of the

[6] It is also found in the mucilage ("gum") cells of tragacanth, as noted by earlier observers.

mucilage cells not only in the cacti but also in the mallows, Tilia, Sterculia, tragacanth (*Astragalus gummifer* Labill), etc., have been comprehended. Kützing (through Wigand, 1863) as early as 1851 saw the lamination in tragacanth, but had an entirely incorrect idea of the origin of the mucilage cells, regarding them as a fungus. Mohl in 1857 (also through Wigand) explained the appearance of gum tragacanth as due to the centripetal deorganization of the cell membranes and their change into the gum, a view which certain later observers (Karsten, Schleiden, Wigand) adopted. Cramer in 1855 saw the lamination of the mucilage cells in the cacti. Wigand (1863, p. 149) described the collapsed protoplasmic utricle as a more or less evident trace of an ill-defined cavity, with radiating arms ("radiations") penetrating the mucilage-content in a manner analogous to pore-canals, without properly apprehending the significance of these details. Walliczek (1893) correctly described in a topographical sense the structure of the cell as a whole, but erred, as I think, with Longo (1896) in regarding the mucilage in Epiphyllum, etc., as granular, the granules (so regarded by Walliczek) being merely cavities (Longo) due to dehydration by alcohol, in which medium Walliczek examined his material. His description of the mucilage cells in *Opuntia Tuna* appears to be incorrect, since "mucilage strands stretched in an irregular network through the lumen" do not occur. It would seem that he misinterpreted one of the bizarre conditions caused by rapid hydration in which there are numerous delicate and meshed strands of protoplasm passing out radially through the mucilage to the cell wall. This appearance I have often seen, and is caused by the pinching of the protoplasm by mutually appressed masses of mucilage. Under such conditions the protoplasm is squeezed into lacunated layers, thus producing the meshed appearance described by Walliczek.

Concerning the mode of origin of the mucilage there are diametrically opposed views, namely: (*a*) that the mucilage is secreted within the protoplasm (Lauterbach, 1889, *fide* Walliczek, p. 267) or in extreme form that the mucilage cells have a "plasma gommeux, qui vie et végète à la manière du plasma des cellules ordinaires" (Trécul, 1862, and restated in 1875); and (*b*) that the mucilage is some form or product of the cell wall. The latter view appears in different forms.

Wigand thought that the mucilage arises by a deorganization of an already present secondarily much thickened cell wall. De Bary (1884, p. 144), following Wigand (whom he cites), and apparently depending on his descriptions, regarded the mass of mucilage in the cells of mallows, cacti, and laurels as having the "structure of a very thick, abundantly and delicately stratified cell wall," and that it is . . . nothing more than a cell wall *which has thickened* [italics mine] strongly at the expense of the central cavity."

Walliczek (p. 268) thought that the mucilage is *laid down on the primary membrane as a secondary thickening*, in the formation of which the primary

cell wall takes no active part, saying specifically that the plasma lays down the secondary wall (mucilage) *on its outer external surface.* He believes that in so stating the case, he agrees with de Bary and not with Wigand.[7] It has however been shown in the previous pages of this paper that the inner portion of the original cell wall is altered into a hydrocellulose at the time when mucilage begins to appear. The mucilage arises therefore by hydrolysis of the original cell wall which shows no striking or excessive secondary thickening, and not by deposition by the plasma on this wall of additional new material, or by the alteration of a thick secondary cell wall, whether laid down as cellulose or as bassorin. Neither Walliczek's view, nor that stated by de Bary, is therefore correct.

This account applies equally to the mallows, cacti, Tilia and tragacanth.[8] The last named I have been able to study only from a fragment of stem opportunely included in a fragment of the gum, and from the gum itself. In agreement with Mohl and Wigand, I found the lamination of the mucilage, and the included starch. I found also fragments of the original cell walls, both of mucilage cells and of non-mucilaginous parenchyma cells. The walls of the mucilage cells bear evidence of extensive hydrolysis, as they are incomplete and show thinned-out edges, while the others show a tearing effect. The protoplasmic utricula with included plastids and starch grains are also very easily identifiable. The original cell walls in the gum are usually very thin and only partially present,[9] and it would seem that in addition to hydrolysis of the cell-wall, that of the middle lamellae must also have taken place in order to bring about the result seen in gum tragacanth, especially in view of the manner of its exudation. This would seem to explain the large amount of "composé pectique," in part pectose, which analyses of gum tragacanth have furnished (Giraud, through Tschirch, p. 399). Bassorin is described as insoluble in water, and is regarded as furnishing only a mechanical suspension as compared with the mucilages of cacti, etc. The distinction is hardly justified. It is true that tragacanth produces an imperfect solvation, the degree depending on temperature, etc., somewhat as in the case of agar-agar, and that in any event there is a lack of homogeneity in the dispersion as compared with one of cactus mucilage. Somewhat the same sort of difference is found on comparing the mucilage of Tilia or Malva with that of Opuntia, the latter yielding a

[7] It is hardly profitable to consider Wigand's views too seriously, since he evidently confused the cytology of the mucilage cells of salep (*Orchis* sp.) with that of the mucilage cells of cacti and mallows (p. 149). Indeed his view quoted above was based on the raphide cells of salep and immediately applied to the cacti—an obviously impossible comparison.

[8] Whether the "gum" of *Sterculia* sp. is to be included with these is doubtful. But the "bassora-gum" studied by Wigand (*l. c.*) showed without any doubt that, whatever its origin, unknown to Wigand, it has the same character as tragacanth. Sterculia gum is said not to show lamination (Maiden, *fide* Tschirch, vol. 2, pt. 1, p. 400).

[9] Just what the thickness of the original walls is in tragacanth I am unable to say. Judging however from the illustrations available (Tschirch), they show no evidence of marked thickening previous to the arising of the mucilage.

much more viscous product. Carnegiea yields a mucilage of low viscosity, quite as low as that of Tilia. Indeed I find like differences between different species of Opuntia. Doubtless a refined technic would discover chemical differences between these various mucilages. Here it is only to say that the distinction between a "gum" and a mucilage is not, at the present, one which corresponds with the manner of origin of these substances. It is pertinent in this connection to remark that Tschirch's organological classification is in a sense inadequate, as it brings into association mucilages of distinctly different kinds: *e. g.*, salep is intercalated between the mallows and tragacanth.

Chemical and Staining Reactions of the Mucilage

The determination of the chemical composition of the mucilage is obviously a problem within the field of biochemistry, and it is, therefore, not my purpose to pass beyond the limits set by the methods I have used.

It has been shown that previous to the occurrence of mucilage, the inner zone of the cell wall gives the reaction of hydrocellulose. The mucilage itself gives no indication of this origin, as with iodine and sulphuric acid it colors only yellow or brownish. It is therefore a cellulose-mucilage if regarded from the point of view of its origin.

It is hydrolyzable by chromic acid, though very considerably more resistant to this reagent than the middle lamella. The tissues may be immersed for several hours in 10% chromic acid, whereby the mucilage cells are set free in their entirety, and, after washing, may be preserved indefinitely in water, and in this condition afford practically unaltered pictures. If the action of the reagent is more prolonged, more or less swelling occurs and consequent rupture beyond the confines of the cell walls. Ultimate complete hydrolysis follows still longer treatment, especially at higher temperatures.

The mucilage is hydrolyzed also by sulphuric, nitric, and hydrochloric acids.

It submits slowly to the digestive action of organisms. The time occupied in materially reducing the viscosity of a solution sufficiently for it to be recognizable to the eye was about six weeks. At the end of eight to ten weeks the viscosity had been lowered to that of water, or nearly so. A second lot, having an initial viscosity several times greater than the above, still shows after six months a considerable viscosity, but much nearer to that of water than to its initial viscosity. During the process a series of odors has been produced, some of which were undoubtedly due to protein putrefaction. The organisms involved are not yet determined.

Staining

No attempt has been made to exhaust the possibilities of staining the mucilage cells in the ordinary sense.[10] For the purpose of demonstrating

[10] For the ordinary methods see Strasburger-Koernicke's Botanisches Practicum; Tunmann; Tschirch.

their distribution and behavior during swelling safranin in alcoholic solution was found very good. Thick sections are first stained and are then allowed to hydrate under the microscope. The total enlargement of the mucilage cells, due to the swelling of the imprisoned mucilage, is shown in the strains on the walls of the neighboring cells. This affords a picture of what may occur in the growing plant when under altered conditions of acidity the volume of the mucilage cells is altered.

Neutral red. If living sections are placed in a very weakly acidified solution (I used acetic acid) of low concentration of the stain, the mucilage *in situ* will slowly take up the stain. Pronounced results may not be expected in less than twenty-four hours. The cell walls also stain, their color being a deeper red as compared with the rose pink of the mucilage, a difference doubtless referable to the degrees of dispersion of the colloidal systems. The annulae of the vessels also stain pink. In a strong solution of the stain the strong coloration of walls and protoplasm and the deep staining of the mucilage itself make observation difficult.

In a slightly alkaline (to neutral red) solution the mucilage is not stained, or at length very slightly, although the included protoplast becomes deeply stained. The cell sap (in living sections) becomes loaded with the stain.

Ruthenium red is taken up vigorously by the mucilage, as also by the cell walls and protoplasm.[11] The stain has a dehydrating effect ont he mucilage, which, when in high enough concentration, is sufficient to prevent swelling.

The above mentioned behavior of mucilage toward neutral red in acid and alkaline solution, and the dehydrating effect of ruthenium red, coupled with other frequent observations of my own which need not be detailed, led me to inquire more particularly into the relation of the mucilage as an emulsion colloid to dyes in general. It may be recalled that I showed some time ago (1911) that tannin is adsorbed by a cellulose-like body when coagulated, and only weakly so when not coagulated. This appears as a parallel behavior to that of cactus and other mucilage toward neutral red. At all events it was evident that the adsorption of a stain by the mucilage is related to the degree of hydration, and on attempting to investigate this relation it was further determined that certain dyes themselves alter the dispersive relations of the mucilage. *E. g.*, it was found that ruthenium red *forms membranes on the surface of a mass of mucilage* (Opuntia, Tilia, etc.) as tannin does on a hydrated albumin. On mixing dyes with mucilage it developed that certain of them *gradually lower the viscosity of the mucilage* till it approaches closely that of water, and that the dyes which are most effective are those which are most strongly adsorbed. The emulsion-

[11] It is rather usual (*e. g.*, Ishikawa, 1918) to quote this reagent as staining the pectic substances, allowing the inference to be drawn that it is specific in this regard. This is, however, not in any sense true, as I have previously observed (1916, p. 219.) Further on this, however, beyond.

colloidal properties are thus quite altered, and the complex takes on the character of a suspension (Lloyd, 1918*b*). By means of alcohol, further dehydration followed by precipitation occurs and the amount of adsorbed stain is reduced, when the mucilage may be recovered as such but with slightly altered physical properties, since it no longer swells indefinitely in water. The following stains have been investigated as to this behavior. They are mentioned in series, beginning with the most vigorously adsorbed and therefore the most active in reducing viscosity. The material was from *Opuntia Blakeana*. Ruthenium red > neutral red > Bismarck brown = gentian violet = methylene blue > safranin > methyl green > erythrosin. The viscosity was unaltered after nine days by fuchsin, methyl blue, coralline, orange G, methyl orange. For the concentrations used, an observable lowering of viscosity followed after twenty-four to forty-eight hours in the case of the most vigorously adsorbed stains. A brief examination of the mucilage of *Ulmus fulva* and of Linum (seed-coat) has indicated that they behave similarly toward ruthenium red and neutral red. Further investigation is in progress.

Conclusions

1. Mucilage in the cacti, mallows, and tragacanth arises within specialized parenchyma cells by hydrolysis of the cellulose wall, *which is not secondarily thickened*. The first visibly demonstrable change is from cellulose to a hydrocellulose; from this the mucilage arises. As this hydrates, it swells and compresses the protoplasm toward the middle of the cell. The protoplasm remains attached more or less to the pits (where little or no hydrolysis of the wall appears to occur), giving rise to radiating strands mimicking the strands within the protoplast extending from the nucleus to the wall-layer.

The mucilage shows lamination which is determined by water content. It is quite possibly predetermined by the apposed layers of cellulose in the original cell wall. The lamination has evidently led certain previous observers to the idea that the mucilage arises as a secondary thickening in the structural sense.

The mucilage is neither laid down as a secondary layer, nor is it secreted within the protoplast, nor yet is it a secretion thrown out as mucilage from the outer surface of the protoplasm.[12]

In tragacanth it appears that the hydrolysis of the cell walls is more extensive than in such forms as Opuntia, Tilia, the mallows, and is at the same time accompanied by digestion of the middle lamella.

2. The mucilage adsorbs certain dyes with great vigor, others with lesser and different degrees of vigor, and still others not at all.

The viscosity of the mucilage is lowered by those dyes which are adsorbed, at a rate and to an extent in direct relation to the degree of adsorption.

[12] See summary in Tunmann (1913).

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